Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 4754–4758, May 1992
Cell Biology

Tumor necrosis factor α is an autocrine growth regulator during macrophage differentiation

(hematopoiesis/macrophage heterogeneity/macrophage growth factors/cytokines)

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Communicated by Marvin P. Bryant, February 10, 1992 (received for review November 26, 1991)

ABSTRACT **Previous experiments have revealed the expression of tumor necrosis factor α (TNF-α) transcripts in all murine bone marrow-derived macrophage colonies isolated from days 5 through 9 of differentiation *in vitro*. These results implicated a role for TNF-α gene expression during macrophage differentiation. Antisense oligomers to the initiation region of the TNF-α message were used to inhibit its expression, thus allowing the role of TNF-α gene expression in controlling the differentiation of macrophages to be determined. Results showed that TNF-α regulated the proliferation of macrophages during differentiation. Cells isolated on day 3 were exclusively vulnerable to the effects of blocking TNF-α gene expression, displaying a 30% increase in proliferation over control cells or sense oligomer-treated cells. Thus, in the absence of TNF-α gene expression, cells maintained proliferation instead of undergoing terminal differentiation. Exogenous TNF-α was capable of rescuing day 3 antisense-treated cells, therefore maintaining normal levels of proliferation. In contrast, blocking interleukin 1β gene expression by antisense oligonucleotide treatment had no effect on proliferation. Addition of exogenous recombinant murine or human TNF-α decreased the total cell number 25–50% regardless of whether cells were grown in medium containing colony-stimulating factor 1 (CSF-1) or granulocyte–macrophage colony-stimulating factor (GM-CSF). These results suggested that exogenous TNF-α suppressed proliferation of early hematopoietic progenitors, whereas endogenous TNF-α regulated proliferation of macrophage progenitors. The number of differentiated, adherent macrophages on day 5 of differentiation *in vitro* was increased by TNF-α treatment of GM-CSF-induced macrophages but was suppressed in CSF-1-induced macrophages. These findings suggest that distinct TNF receptor expression and/or signaling is induced in differentiating macrophages stimulated with either growth factor.**

In addition to the nonspecific tumoricidal activity of activated macrophages, tumor necrosis factor α (TNF-α) is known to modulate monocyte function and induce monocytic differentiation in some myeloid cells lines (reviewed in ref. 1). Treatment of the monocytic cell line HL-60, a human promyelocytic leukemia cell line, with low doses of TNF-α for 6 days induced partial differentiation as judged by the acquisition of a differentiated phenotype. TNF-α has been shown to be identical to differentiation-inducing factor, a T-cell lymphokine capable of inducing differentiation of human ML-1, HL-60, and THP-1 myeloid cells (2). Both differentiation-inducing factor and recombinant human TNF-α (rhTNF-α) induced maturation of ML-1 cells along the monocytic path, as defined by the acquisition of abundant granules in cytoplasm, eccentric oval nucleus, and esterase stain. Activities ascribed to TNF-α were neutralized with antibodies to differentiation-inducing factor (2). Furthermore, rhTNF-α treatment was capable of a dose-dependent inhibition of leukemic colony and granulocyte–macrophage colony formation (3–5). Similarly, pulsing bone marrow cells with TNF-α inhibited colony formation, although murine cells were less sensitive to inhibition by rhTNF-α than were human cells (3). The inhibitory effect of TNF-α was demonstrated to be most profound on cells cultured in granulocyte colony-stimulating factor and some sources of granulocyte–macrophage colony-stimulating factor (GM-CSF), and the growth inhibition could be abrogated by neutralizing antibodies to TNF-α (4, 5).

Further evidence has suggested that TNF-α also exerts its effect(s) on normal macrophage differentiation. TNF-α treatment of human bone marrow cells did not decrease viability at 24–72 hr of differentiation *in vitro* but led to significant decreases in cell number and increases in differentiated macrophages by 96 hr of TNF-α treatment (6). Our previous studies have demonstrated that TNF-α transcripts were present in all BALB/c bone marrow-derived macrophage (BMDM) colonies isolated on days 5–9 of differentiation, whether cell growth was stimulated by colony-stimulating factor-1 (CSF-1) or GM-CSF (7, 8). Furthermore, endogenous production of TNF-α protein and mRNA has been demonstrated in human HL-60 cells stimulated to differentiate with phorbol esters (3, 9).

Vitamin D_3, TNF-α, and γ interferon (IFN-γ) have been shown to cause human HL-60 cells to differentiate along the monocytic pathway. A direct correlation has been drawn that couples the sphingomyelin cycle with the induction of HL-60 cell differentiation toward the monocytic lineage (10). Sphingomyelin has been shown to undergo significant turnover in response to both TNF-α and vitamin D_3 but peaked much earlier after TNF-α treatment. Agents causing monocyte-like and granulocytic differentiation did not induce sphingomyelin turnover as did TNF-α and IFN-γ, suggesting that this mechanism was a macrophage-related pathway (10). Eukaryotic initiation factor 4E, a cap binding protein involved in the G_1–S transition, was phosphorylated immediately after TNF-α treatment of the human monocytic leukemia cells, U-937, suggesting that TNF-α may be an important differentiation-inducing activity in these cells (11). These observations supported a role for TNF-α expression during macrophage differentiation.

Experiments presented herein demonstrate that TNF-α is an endogenously produced autocrine growth factor that regulates its own differentiation-promoting ability. Antisense

Abbreviations: TNF-α, tumor necrosis factor α; mTNF-α or hTNF-α, murine or human TNF-α; rmTNF-α or rhTNF-α, recombinant mTNF-α or hTNF-α; BMDM, bone marrow-derived macrophage; IL-1β, interleukin 1β; CSF-1, colony-stimulating factor 1; GM-CSF, granulocyte–macrophage colony-stimulating factor; rmGM-CSF and rhGM-CSF, recombinant murine and human GM-CSF; IFN-γ, γ interferon; PGE_2, prostaglandin E_2.

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oligomer treatment was used to block TNF-α gene expression during differentiation. Thus, if TNF-α were an autocrine differentiation factor, in the absence of TNF-α gene expression the cells would follow a proliferative program instead of a differentiative one. Effects on cell proliferation were tested on BALB/c bone marrow cells grown in the presence of either CSF-1 or GM-CSF. Results showed that blocking TNF-α gene expression during a specific window of differentiation caused the cells to lose their growth-regulating ability, thus resulting in uncontrolled growth.

Two receptors for TNF have been cloned and can be distinguished by the species specificity of their TNF-α binding (12, 13). The 75- to 80-kDa receptor (p80) specifically binds murine TNF-α (mTNF-α), whereas the 55- to 60-kDa receptor (p60) binds either human TNF-α (hTNF-α) or mTNF-α. To distinguish which of the two receptors was responsible for the growth-inhibitory and differentiation-inducing activities of TNF-α, BALB/c BMDM were cultured in medium containing either CSF-1 or GM-CSF as hematopoietic stimulus in the presence of mTNF-α or hTNF-α. Results suggested that BMDM cultured with CSF-1, which we call "CSF-1-derived BMDM," were inhibited more from proliferation and differentiation by mTNF-α than by hTNF-α, implicating p80 as the responsible receptor. In contrast, BMDM cultured with GM-CSF, which we call "GM-CSF-derived BMDM," were induced to differentiate in response to either hTNF-α or mTNF-α, suggesting p60 was responsible for differentiation-inducing activities.

MATERIALS AND METHODS

Cell Culture. Six- to 10-week-old mice were obtained from Harlan–Sprague–Dawley. Bone marrow plugs were harvested from BALB/c femurs in Hank's balanced salt solution, dispersed into a single cell suspension, and washed. Culture medium consisted of Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal bovine serum, 10% (vol/vol) horse serum, 0.075% sodium bicarbonate, 5 mM Hepes buffer, 2 mM L-glutamine, penicillin at 10,000 units/ml, streptomycin at 10 μg/ml, and 1× Sigma vitamin mix. Either recombinant murine GM-CSF (rmGM-CSF) (200 units/ml; Immunex, Seattle) or L929 supernatants (source of CSF-1; 200 units/ml by proliferation assay) were used as the growth factor. Cultures were maintained in a humidified chamber at 37°C in 7.5% CO_2/92.5% air.

Antisense Oligomers. Crude oligomers (17 base pairs) specific to the initiation region of the TNF-α message (TNF-α sense, 5'-CACAGAAAGCATGATCC-3'; TNF-α antisense, 5'-GTGTCTTTCGTACTAGG-3') were obtained from the Genetic Engineering Facility, University of Illinois. Oligomers specific to the initiation region of the interleukin 1β (IL-1β) message. (IL-1β sense, 5'-ATGGCAACTGTTCCTGA-3'; IL-1β antisense, 5'-TACCGTTGACAAGGACT-3') were obtained from Operon Technologies (Alameda, CA).

RNA Isolation and Northern Blot Analysis. BALB/c bone marrow cells (5×10^7 to 1×10^8 cells) were grown in medium containing rmGM-CSF (200 units/ml). Cells were treated with medium or 5 μM sense or antisense TNF-α oligomers 20 hr prior to harvest on day 3 of differentiation *in vitro*, and total RNA was isolated by the guanidinium isothiocyanate method (14). RNA was size-fractionated by electrophoresis through a 1% agarose gel containing 0.02 M Mops buffer and 2.2 M formaldehyde and was transferred to Biodyne A nylon membranes (VWR Scientific) by using a Bio-Rad Trans-Blot apparatus. The immobilized RNA was hybridized with a ^{32}P-labeled TNF-α DNA probe, the *Eco*RI/*Pst* I insert from plasmid pGEM-3-cach (a gift from B. Beutler, University of Texas Southwestern Medical Center, Dallas). The filter was washed at room temperature for 15 min in 2× SSPE (1× SSPE = 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA) containing 0.1% SDS, followed by two 30-min washes at 58°C in 0.1× SSPE containing 0.1% SDS, and was exposed to XRP-5 film with intensifying screen.

The photograph of the ethidium bromide-stained agarose gel and the autoradiogram were compared by scanning laser densitometry (Molecular Dynamics, Sunnyvale, CA) to verify consistency of loading.

TNF-α Secretion Assay. BALB/c bone marrow cells were grown for 3 days in medium containing GM-CSF (200 units/ml) or CSF-1 (200 units/ml). Both sense and antisense oligomers (5 μM) were added to cells along with 100 ng of bacterial lipopolysaccharide per ml 6 hr prior to harvest. Supernates were collected, and cellular debris was pelleted by centrifugation at 12,000 × g. TNF-α cytotoxic activity was measured as described (15). Plates were read at 540 nm in a microplate reader. Activity was determined by the following equation:

$$\% \text{ cytotoxicity} = \frac{A_{\text{spontaneous}} - A_{\text{sample}}}{A_{\text{spontaneous}} - A_{\text{total}}} \times 100.$$

One unit of TNF-α was defined as the reciprocal of the dilution causing 50% cell killing.

Proliferation Assays. Bone marrow cells were plated in the cell culture medium described above at 10^5 cells per 200 μl in 96-well plates. Triplicate assays were performed with medium alone (control) or with 5 μM sense or antisense oligomers. When indicated rmTNF-α (100 units/ml; Genzyme) was incubated with cells for 18 hr prior to harvest. Cell aliquots were diluted 50% in 0.4% trypan blue, and cells were counted with a hemocytometer.

TNF-α Effects on Differentiating BMDM. Bone marrow cells were plated in triplicate at 5×10^5 nucleated cells per ml in 24-well plates. CSF-1 or rmGM-CSF at 200 units/ml or a combination of both (each at 100 units/ml) was used as the hematopoietic stimulus in the presence or absence of 200 units of rmTNF-α or rhTNF-α per ml (National Institute for Biological Standards and Control, Hartfordshire, U.K.). Cells were harvested at day 5 of differentiation *in vitro*, and both the total cell number and adherent cell number were determined by counting on a hemocytometer.

Statistical Analysis. Statistical analyses were performed by using a two-tailed Student t test. Each experiment was performed in triplicate, and the mean ± SEM is shown.

RESULTS

Cell Viability after Oligomer Treatment. Cells were titrated with up to 10 μM sense or antisense oligomer, and viability was determined by propidium iodide staining and flow cytometric analysis (Table 1). The lowest concentration of oligomer displaying the maximum induction of proliferation was determined to be 3 μM (data not shown). This concentration of oligomer is in agreement with several studies showing the uptake and stability of oligomers in monocytes and myeloid progenitor cells (16, 17).

Determination of TNF-α Production by BMDM After Antisense Treatment. TNF-α message levels were determined by Northern blot analysis after 20 hr of antisense oligomer (5 μM) treatment of GM-CSF (200 units/ml)-derived cells. Fig. 1 shows the decreased level of TNF-α transcripts (50% decrease in transcripts as determined by scanning laser densitometry) after treatment of the cells with antisense TNF-α oligomers, whereas the sense oligomer exerted no effect. Mature TNF-α protein was also measured after 6 hr of incubation with the antisense oligomer. Bone marrow cells were grown separately in medium containing CSF-1 or GM-CSF at 200 units/ml. Cells were treated with bacterial

Table 1. Effect of oligonucleotide concentration on BMDM viability and TNF-α-induced cytotoxic activity

	Hematopoietic stimulus at 200 units/ml			
	GM-CSF		CSF-1	
Oligomer (μM)	Viability,* %	Cytotoxicity†	Viability,* %	Cytotoxicity†
Control	82.2	416 (100%)	61.8	48 (100%)
Sense (1.0)	83.9	ND	ND	ND
Antisense (1.0)	84.9	ND	ND	ND
Sense (10.0)	82.6	307 (26%)	71.3	ND
Antisense (10.0)	84.8	107 (74%)	64.5	30 (38%)

ND, not determined.

*Oligomers were added 18 hr prior to harvest on day 3. Viability in percent was determined by flow cytometry of propidium iodide-stained samples.

†TNF-α-induced cytotoxic activity was determined as described in *Materials and Methods*. One unit is defined as the reciprocal of the dilution causing 50% cell killing. Numbers in parentheses represent percent inhibition of cytotoxic activity.

lipopolysaccharide at 100 ng/ml to induce TNF-α production 6 hr prior to harvest. Simultaneously, either the sense or antisense oligomer was added to the cultures. The level of TNF-α cytotoxic activity was decreased by the antisense oligomer ≈74% and ≈38% in GM-CSF- and CSF-1-derived cells, respectively (Table 1).

Time Course for Blocking TNF-α Gene Expression. The susceptibility of BMDM to a block of TNF-α gene expression was dependent upon the state of maturation of the cells. Oligomers were added to cells 18–24 hr prior to harvest, and total cell numbers were determined. The maximal effect of inhibition of TNF-α gene expression occurred on day 3 of *in vitro* differentiation, whereas addition of oligomers on other days had no effect (Fig. 2). This suggested that as the GM-CSF-derived BMDM differentiated toward the macrophage lineage, they passed a window in which they were profoundly responsive to the endogenous production of TNF-α. Thus, if TNF-α expression were obliterated, the cells lost a control mechanism and they maintained proliferation rather than differentiating.

Growth Factor-Induced BMDM Proliferation in Response to TNF-α or IL-1β Abrogation. Table 2 shows the effect of endogenous and exogenous TNF-α on differentiating

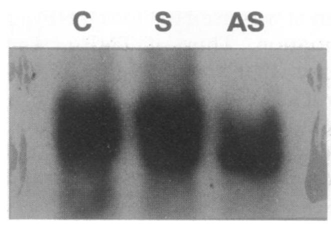

FIG. 1. Effect of TNF-α antisense oligomers on TNF gene transcripts: inhibition of TNF-α mRNA accumulation. BMDM were isolated on day 3 of differentiation *in vitro* for Northern blot analysis for TNF-α message. During the final 20 hr of culture, cells were treated with media alone (lane C), with sense oligomers (5 μM) (lane S), and with antisense oligomer (5 μM) (lane AS) to the TNF-α message.

BMDM. Blocking TNF-α gene expression with antisense oligomers resulted in a 30% increase in proliferation exclusively in GM-CSF (200 units/ml)-derived BMDM. Furthermore, the addition of rmTNF-α (100 units/ml) was capable of rescuing the TNF-α antisense-treated cells from increased proliferation. Inhibition of TNF-α expression at two GM-CSF concentrations (200 and 2000 units/ml) showed that TNF-α functioned in a macrophage-specific autocrine pathway (Table 2). At GM-CSF concentrations <200 units/ml, the effect of antisense TNF-α oligomers on proliferation was minimal. This was probably due to the lack of stimulation of proliferation at these concentrations (data not shown). At the lower concentration of GM-CSF (200 units/ml), which predominantly induces macrophage differentiation, the degree of proliferation was increased ≈30% over control values. Bone marrow cells derived from culture with the high concentration of GM-CSF—2000 units/ml, the concentration inducing granulocytic formation—resulted in no statistically significant increases in proliferation in response to treatment with antisense TNF-α. Furthermore, CSF-1 (200 units/ml)-derived BMDM displayed no effect on proliferation after inhibition of TNF-α expression (Table 2).

Molecular phenotyping experiments have shown that IL-1β, like TNF-α, was expressed in a high percentage of BMDM colonies (8). Thus, experiments were undertaken to examine whether TNF-α imposed a specific effect on macrophage differentiation or whether IL-1β shared in this effect, since it also was highly expressed. Antisense oligomers to the

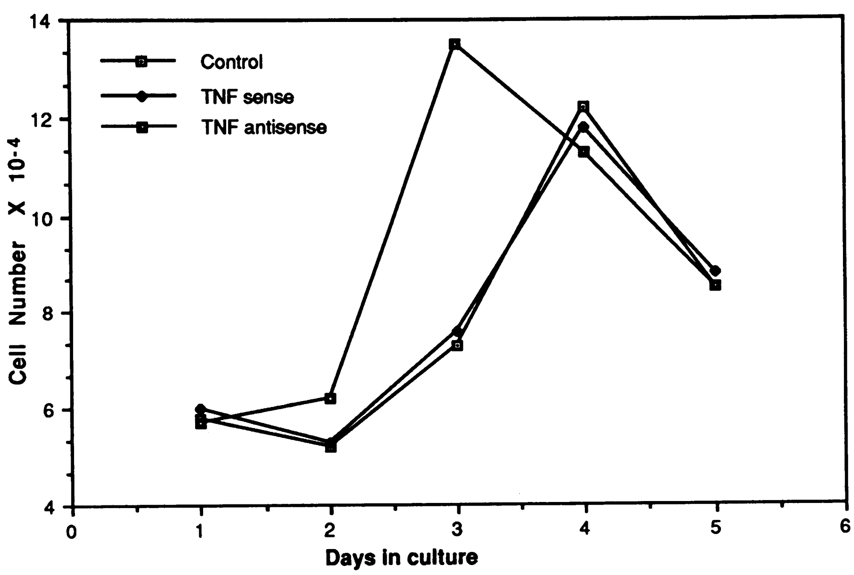

FIG. 2. Time course of TNF-α antisense treatment on proliferation of BMDM. Cells were seeded at 10^5 per 200-μl well. Media or 5 μM sense or antisense TNF-α oligomers were added to cells 18 hr prior to cell counting. Cells in wells were counted in triplicate daily for each treatment. Results are presented for one of two representative experiments with similar results.

Table 2. Effects of TNF-α TNF-α and IL-1β on BMDM proliferation

	Augmentation of cell growth, % proliferation		
Treatment	rmGM-CSF* (200 units/ml)	rmGM-CSF† (2000 units/ml)	CSF-1‡ (200 units/ml)
Control	100	100	100
TNF-α sense	101 ± 4.0	105 ± 6.0	92 ± 4.0
TNF-α antisense	127 ± 6.0§	114 ± 24.0	93 ± 4.0
IL-1β sense	94 ± 1.0	96 ± 3.0	98 ± 3.0
IL-1β antisense	96 ± 7.0	104 ± 5.0	90 ± 9.0
rmTNF-α	70 ± 10.0§	52 ± 9.0§	76 ± 9.0§
TNF-α antisense + rmTNF-α	88 ± 9.0	59 ± 3.0§	90 ± 7.0

Day 3 BMDM were treated 18 hr prior to cell counting with sense or antisense oligomers to TNF-α or IL-1β (5 μM); when indicated, rmTNF-α (200 units/ml) was added 18 hr prior to cell counting. Experiments were performed in triplicate.

*Results compiled from 12 representative experiments.

†Results compiled from 5 representative experiments.

‡Results compiled from 4 representative experiments.

§Value significantly different from control value ($P < 0.01$).

initiation region of IL-1β were used to block its expression during differentiation. Inhibition of IL-1β expression had no effect on the proliferation of BMDM induced to differentiate with either GM-CSF at 200 or 2000 units/ml or CSF-1 at 200 units/ml at day 3.

TNF-α Effects on Differentiating BMDM. The effects of rhTNF-α and rmTNF-α on BMDM proliferation were examined to address the role that each of the TNF receptors, p60 and p80, play in macrophage differentiation. After derivation in the presence of either source of TNF-α, total cell numbers were decreased for both CSF-1- and GM-CSF-derived BMDM with slightly more inhibition from rmTNF-α than rhTNF-α on day 5 of differentiation (Table 3). Similarly, the number of differentiated, adherent cells was decreased to the same extent as the total cell population in CSF-1-derived cells. In contrast, BMDM cultured in the low concentration of GM-CSF (200 units/ml) displayed an increase in differentiated cells to the same degree by addition of either rhTNF-α or rmTNF-α. Adherent cell numbers from BMDM induced by the high concentration of GM-CSF (2000 units/ml) were not affected by the addition of rhTNF-α or rmTNF-α. The most striking increase in the percentage of differentiated cells was in the group induced by a combination of CSF-1 and GM-CSF, although the total number of adherent cells was somewhat lower than the number of cells derived in GM-CSF alone.

Table 3. Effects of hTNF-α or mTNF-α on day 5 BMDM differentiation

Hematopoietic* growth factor (units/ml)	Treatment	Cell number (%) Total†	Adherent
CSF-1 (100)	Medium	5.6 ± 0.3 (100)	4.8 ± 1.0 (100)
	rhTNF-α	4.3 ± 0.3 (77)	3.7 ± 0.6 (77)
	rmTNF-α	3.1 ± 0.6 (55)	2.5 ± 0.1 (52)
GM-CSF (200)	Medium	10.6 ± 0.4 (100)	5.4 ± 0.6 (100)
	rhTNF-α	7.0 ± 0.6 (67)	7.1 ± 0.9 (131)
	rmTNF-α	6.9 ± 0.8 (65)	7.3 ± 0.5 (135)
GM-CSF (2000)	Medium	ND	5.1 ± 0.1 (100)
	rhTNF-α	ND	5.2 ± 0.0 (102)
	rmTNF-α	ND	4.5 ± 0.5 (88)
CSF-1 (100)/ GM-CSF (100)	Medium	10.4 ± 1.1 (100)	1.2 ± 0.3 (100)
	rhTNF-α	7.1 ± 0.3 (68)	3.7 ± 0.2 (308)
	rmTNF-α	7.1 ± 0.4 (68)	3.1 ± 0.5 (258)

ND, not determined.

*BMDM were cultured for 5 days in medium containing the indicated growth factors alone or in the presence of rhTNF-α or rmTNF-α at 200 units/ml prior to counting total and adherent cell numbers.

†Mean cell number × 10^5 ± SEM was determined for triplicate wells by using a hemocytometer. Numbers in parenthesis represent the percent proliferation. Results are presented for one of two representative experiments with similar results.

DISCUSSION

Autocrine growth-factor production has been associated with pathological conditions such as tumorigenesis (18). Gene-transfer techniques have been used to induce autocrine growth-factor production in immortalized cell lines, thus rendering them completely transformed and tumorigenic. However, the mere induction of autocrine stimulation of primary cells has not led to cellular transformation. Furthermore, autocrine growth-factor stimulation has been shown to regulate cellular proliferation in both normal endothelial cells and the HL-60 cell line (19, 20).

Here we demonstrate that incubation of bone marrow cells with antisense oligomers to TNF-α resulted in decreased TNF-α message and cytotoxic activity but increased the proliferation of GM-CSF-derived BMDM. The decreased TNF-α expression was presumably due to degradation of the message by RNase H and inhibition of translation of the RNA·DNA duplex (21). Blocking TNF-α expression during differentiation of BMDM resulted in increased proliferation only on day 3 of differentiation *in vitro*. These results suggest the involvement of an autocrine mechanism in which TNF-α expression on days 2 and 3 of macrophage differentiation *in vitro* signals the onset of differentiation and the cessation of proliferation. Although IL-1β has been shown to be expressed by a high proportion of BMDM colonies, blocking its expression did not effect the proliferative capacity of differentiating BMDM (8).

Exogenous TNF-α itself is a potent inhibitor of hematopoietic cell proliferation (3–5). Addition of exogenous TNF-α decreased total cell proliferation in cultures with all growth factors tested, but, interestingly, GM-CSF-derived cells were induced to differentiate as judged by an increase in the number of adherent cells (Table 3). These experiments agree with work by Srivistava *et al.* (6) in which an increase in adherent monocytes was detected after 96 hr of rhTNF-α treatment of GM-CSF-induced human bone marrow cultures. It is interesting that CSF-1-derived cells displayed no such effect, as the number of differentiated cells was inhibited in parallel with the total cell population. This is in contrast to work in which hTNF-α was shown to double the proliferative response of CSF-1-derived BMDM (22). These studies also showed a 10-fold decrease in CSF-1 binding sites after TNF-α administration to BMDM (22, 23). The discrepancies observed between the results shown here and those of Branch *et al.* (22) and Shieh *et al.* (23) are probably attributable to the state of maturation of the cells or the level of TNF-α used, or both. Our experiments utilized immature macrophage progenitors to measure the degree of induced differentiation, whereas Shieh *et al.* (23) used peritoneal macrophages and Branch *et al.* (22) used day 7 BMDM and high levels of hTNF-α (1,250–16,000 units/ml). Both peritoneal macrophages and day 7 BMDM are mature, differentiated macrophage populations and may be under distinct regulatory mechanisms as compared with differentiating progenitor cells.

The 75- to 80-kDa TNF receptor (p80) is species specific, reacting only with mTNF-α whereas the 55- to 60-kDa receptor (p60) is not species specific. The p60 receptor sequence is thought to be identical to the TNF inhibitor purified from serum and urine (13, 24, 25). Moreover, the purified TNF inhibitor has been shown to be capable of inhibiting TNF-α-induced prostaglandin E_2 (PGE_2) production by dermal fibroblasts in a dose-dependent fashion (26).

The apparent species specificity of TNF-α, which has been observed in some but not all circumstances, has been attributed to the receptor involved in the TNF-α actions. The 60-kDa receptor displays crossreactivity with mTNF-α and hTNF-α, whereas the 80-kDa receptor is species specific (12, 13). This information allowed us to propose a mechanism for the preferential induction of monocytic differentiation of GM-CSF-derived BMDM as shown in Table 3. CSF-1-derived BMDM were primarily inhibited from proliferating and differentiating by rmTNF-α. This would suggest that p80, which is specific for mTNF-α, was coupled to the inhibitory actions of TNF-α in these cells. However, BMDM cultured with GM-CSF or a combination of GM-CSF and CSF-1 displayed a decrease in total cell proliferation with a concomitant increase in adherent, differentiated cells. This effect was observed to the same degree in response to both rhTNF-α and rmTNF-α, providing evidence for signaling through p60.

Northern blot analysis and flow cytometry were utilized to determine whether the responses we attributed to p60 and p80 were simply due to expression of these two receptors. Expression of both p60 and p80 mRNA appeared identically regulated in the CSF-1- and GM-CSF-derived populations. Surface expression of p60, as determined by flow cytometry with phycoerythrin-conjugated rmTNF-α, demonstrated low but similar expression of p60 on both populations throughout differentiation (unpublished data). Hence, we conclude that signaling mechanisms, and not simply receptor expression, were responsible for the differences in responsiveness to hTNF-α and mTNF-α. Other studies have similarly concluded that, in spite of differential surface expression of TNF receptors, signaling via the receptors appears to be the controlling factor (27).

These results suggest a feedback control of monocytic differentiation by TNF-α that enhances differentiation of macrophage progenitors and, thus, may help to control cellular reactions during infection. Exposure to GM-CSF and TNF-α, which are released in times of immunologic stress, would induce production of GM-CSF-derived cells that would be under strict regulation by TNF-α. These cells would be induced to differentiate and, thus, perform their specific functions, whereas CSF-1-derived cells would be inhibited from differentiating. Furthermore, immature CSF-1-derived cells could encounter circulating GM-CSF, transforming them into a TNF-α-responsive phenotype similar to the GM-CSF-derived population. Experiments have demonstrated that CSF-1-derived cells are more responsive than GM-CSF-derived BMDM to PGE_2-induced suppression of lipopolysaccharide-stimulated TNF-α production (28). Taken together these experiments would suggest an inflammatory GM-CSF-derived macrophage population and a steady-state CSF-1-derived population. During immunologic challenge, the GM-CSF-derived population would be prominently produced by the bone marrow. As TNF-α was released from the tissues, it would induce differentiation of the GM-CSF-derived cells and inhibit production of the CSF-1-derived cells. Inflammatory lesions are abundant in PGE_2; in its presence GM-CSF-derived cells would be capable of TNF-α secretion, whereas CSF-1-derived cells would be suppressed (28). Moreover, the shed p60 TNF-α receptor (shown to be a TNF inhibitor; ref. 25), presumably expressed exclusively by the GM-CSF-derived population, would be able to inhibit tissue production of PGE_2, thus regulating the inflammatory site. As the challenge subsided, GM-CSF would decrease to normal levels, allowing the steady-state macrophage population to return to equilibrium.

We extend our most sincere gratitude to Dr. S. Gillis at Immunex for the rmGM-CSF, Dr. C. Reynolds for rhTNF-α, and Dr. B. Beutler for the plasmid pGEM-3-cach. We also greatly appreciate the excellent technical assistance of Mark Teutsch and thank Helen Gawthorp for extraordinary work in preparation of this manuscript.

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